THE LIPIDS IN COLPIDIUM CAMPYLUM

CHARLES G. WILBER AND GERALD R. SEAMAN

Biological Laboratory, Fordham University, New York

INTRODUCTION

Various investigators have reported that *Colpidium* contains fat in the cytoplasm. Mast (1938) holds that it is neutral fat; Wilber (1942) maintains that it is chiefly fatty acid. Both investigators, however, base their conclusions on the staining of colpidia with Nile blue sulfate.

In the literature there is apparently no information based on microchemical analyses concerning the formation and types of lipids in *Colpidium*. An investigation was consequently undertaken to ascertain the types of lipids present and the manner of formation. This present report is the first in a series and it deals with the synthesis of lipids in *Colpidium campylum*.

MATERIAL AND METHODS

A sterile culture of colpidia was established (Glaser and Coria, 1930) and was cultivated in 3 per cent Difco proteose-peptone solution (Elliot, 1933). The proteose-peptone was rendered fat-free by extracting it with hot alcohol (Bloor, 1943, p. 40). Solutions of this fat-free medium were negative to all the tests for lipids. Colpidia were washed 3 times in fat-free proteose-peptone solution before transfer from ordinary to fat-free culture medium.

The population of the cultures was estimated by counting the number of colpidia in a small, weighed drop of medium and from that result calculating the number of organisms in 1 cc.

Five days after transfer (when the population reached about 44,000 colpidia per cc.) 1 cc. of the culture (fluid plus organisms) was transferred to 17 cc. of boiling alcohol-ether mixture and the whole was then made up to 25 cc. The alcohol-ether mixture extracted all the lipids (Bloor, 1943). Phospholipids were estimated colorimetrically (Youngburg and Youngburg, 1930), cholesterol colorimetrically (Bloor, 1916), and fatty acid by oxidation (Bloor, 1928). The procedure is somewhat as follows: For estimation of neutral fat and bound cholesterol the extracted material is saponified; this sets free the cholesterol and the fatty acids contained in fat and cholesterol esters. Tests for free cholesterol were made and were consistently negative. Since no cholesterol was found, the fatty acid values represent fatty acid combined in fat and/or free in the extract. It should be remembered that "the cholesterol esters are normally present only in traces. Hence, after phospholipids are separated, the fatty acids present may be safely referred to as fats." (See Bloor, 1943, pp. 42–43.)

A second sample from the same culture was carefully filtered 3 times through fat-free cotton waste and hard filter paper to remove all the colpidia. The filtrate was then analyzed for lipid material.

The differences between the values for the whole culture and for the filtrate were equal to the amounts of lipids in the colpidia themselves.

It is extremely rare that pure fats are found in microorganisms. Usually they are found as mixtures which are almost impossible to separate. "Analyses, therefore, are restricted usually to the fatty acids present and the determination of the ratio of saturated to unsaturated fatty acids" (Porter, 1946, p. 409). This method was followed in the present work, although the degree of saturation of the fatty acids was not measured.

RESULTS

All tests for cholesterol were negative. Table I shows that there is consistently about .0002 mg. of lipid phosphorus per 1,000 colpidia. The table also shows that there is more lipid phosphorus in the culture fluid (2.5 mg./100 cc.) than in the colpidia (0.9 mg. in the colpidia from 100 cc.).

TABLE I

Table showing the amount of lipid phosphorus in the culture medium and in the colpidia

In order to convert lipid *P* to phospholipid (assuming it all to be lecithin) multiply the lipid *P* values by 25.

Culture	Colpidia per cc.	Lipid *P* in whole culture. Mg./100 cc.	Lipid *P* in fluid alone. Mg./100 cc.	Lipid *P* in colpidia from 100 cc. Mg.	Lipid *P* in 1,000 colpidia. Mg.
101	44,000	3.6	2.3	1.3	0.0003
102	47,000	3.3	2.3	1.0	0.0003
103	39,000	3.3	2.6	0.7	0.0002
104	47,000	3.6	2.8	0.8	0.0002
105	44,000	3.1	2.3	0.8	0.0002
Mean	44,000	3.4	2.5	0.9	0.0002

TABLE II

Table showing the amount of fatty acid in the culture medium and in the colpidia themselves

Culture	Colpidia per cc.	Lipid in culture. Mg./100 cc.	Lipid in fluid alone. Mg./100 cc.	Lipid in colpidia from 100 cc. Mg.	Lipid in 1,000 colpidia. Mg.
101	44,000	212	150	62	0.012
102	47,000	225	187	38	0.008
103	39,000	225	175	50	0.013
104	47,000	263	213	50	0.010
105	44,000	237	188	49	0.011
Mean	44,000	232	183	49	0.011

Table II shows that there is consistently about 0.011 mg. of fatty acid per 1,000 colpidia. This table also shows that there is much more fatty acid in the culture fluid (183 mg./100 cc.) than in the colpidia (49 mg. in the colpidia from 100 cc.).

DISCUSSION

At first sight the value of 0.011 mg. of fatty acid in 1,000 colpidia seems large. Calculations were made to ascertain roughly the percentage of the wet weight which is lipid. It was assumed that *Colpidium* is a prolate spheroid. The volume of the individual organism is then expressed: $V = 4/3\pi a^2 b$, in which a is the minor semi-axis and b the major semi-axis.

The mean length of 20 colpidia selected at random from the cultures was 0.064 mm., the mean thickness 0.036 mm. The minor and major semi-axes are then 0.018 mm. and 0.032 mm., respectively. Substituting these values in the equation above we get $V = 0.0000418$ mm^3 for a single colpidium. For 1,000 colpidia, $V = 0.0418$ mm^3.

Assuming the specific gravity of protoplasm to be 1.00, the weight of 1,000 18 colpidia is 0.0148 mg. The amount of lipid is then 24 per cent of the wet weight.

For the first time quantitative estimates of the kind and the amount of lipid in *Colpidium* are presented. The apparent absence of cholesterol is in accord with other investigations on the protozoa (Wilber, 1946 a and b). The evidence strongly suggests that colpidia do not synthesize cholesterol. Whether they assimilate cholesterol, if it is present in the culture medium, is not known. The high content of fatty acid is in accord with results obtained with other micro-organisms: the lipid in the diphtheria bacillus is mostly fatty acid (Chargaff, 1933); all the lipid in the typhoid bacillus is fatty acid (Akasi, 1939); in myobacterium fatty acids make up about 70 per cent of the fat (Pangborn, Chargaff, and Anderson, 1932).

The production of fat (sudanophil granules) from ingested starch and protein has been reported in various ciliates (Zingher, 1933). However, neither fat free nor sterile culture media were used in the investigations.

It is interesting to observe that the colpidia produce such large amounts of lipid that some is eliminated into the surrounding culture fluid. In the present experiments the ratio of *phospholipid in the whole culture/phospholipid in culture fluid alone* is 1.2 and that of *fatty acid in the whole culture/fatty acid in the culture fluid alone* is 1.3. The inverse ratios are 0.7 and 0.8 respectively. All the cultures used were approximately the same age and contained about the same density of population. It is, therefore, not possible at present to conclude whether these ratios are constant or whether they vary with age of culture and size of population. Such an investigation is now in progress.

In the higher organisms, it is known that fat is formed from protein (Atkinson, Rapport, and Lush, 1922; Eckstein, 1929). The present results indicate that there is a synthesis of lipid from protein in *Colpidium* also. The medium in which the organisms were grown contained no measurable lipids. The cultures were completely sterile, as indicated by regular bacteriological tests. After colpidia had grown in the fat-free medium there was appreciable lipid material in the organisms and in the culture fluid. The only source of food for the colpidia was protein (the proteose-peptone). It seems clear, therefore, that the growing colpidia broke down the supplied protein and reformed it into the lipids which were demonstrated by chemical analysis.

SUMMARY

1. *Colpidium campylum* was established in sterile culture and grown in fat-free proteose-peptone solution.

2. The colpidia and the culture fluid in which they were grown were analyzed for fatty acid, cholesterol, and phospholipid.

3. No cholesterol was found. Large amounts of fatty acid were found in the organisms and in the ambient culture fluid. Phospholipid was found in the colpidia and in the culture fluid. There was more lipid in the culture fluid than in the organisms.

4. It is concluded that *Colpidium* synthesizes lipid from protein.

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